cally assessed for EBV infection for 3 years had little effect on the frequency of silent seroconversion but greatly reduced the frequency of IM (20). This is an important result because it suggests that a vaccine to prevent IM might actually work and also because it points the way to the most sensible vaccine strategy, namely to try to prevent IM but not the normal silent infection with EBV. One can easily envisage how a partially effective gp350 vaccine might prevent IM but not silent EBV infection—if a high viral dose proves to be required for the development of IM, the immune response induced by the vaccine might be able to neutralize most of this virus (preventing IM) but not completely protect against infection. There is therefore an increasing case for further efforts to develop an EBV vaccine that could be given to EBV-seronegative teenagers or adults to try to prevent the development of IM.

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Overstaying their welcome: defective CX3CR1 microglia eyed in macular degeneration

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Age-related macular degeneration (AMD), the most common cause of blindness in the elderly, is characterized by degeneration of the macula and can lead to loss of fine color vision. Alterations in inflammatory and immune system pathways, which arise from genetic differences, predispose individuals to AMD. Yet the mechanism of disease progression with respect to inflammation is not fully understood. In this issue of the JCI, the study by Combadière and colleagues shows that CX3C chemokine receptor 1–deficient (CX3CR1-deficient) mice have abnormal microglia that accumulate beneath the retina and contribute to the progression of AMD (see the related article beginning on page 2920).

Nonstandard abbreviations used: AMD, age-related macular degeneration; C62, CC chemokine ligand 2; Ccr2, CC chemokine receptor 2; CX3CL1, CX3C chemokine ligand 1; CX3CR1, CX3C chemokine receptor 1; RPE, retinal pigment epithelium.

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CX3CR1-deficient mice, microglia accumulate in the subretinal space, evoking morphological and pathological features similar to those observed in human AMD. The data suggest an important role of CX3CR1 and of microglia in the pathogenesis of AMD.

The retina functions as film in a camera
The retina is composed of several transparent neuronal layers. Light focused through the cornea and lens passes across these layers to interact with the pigments in the photoreceptor outer segments, causing a chemical transformation. This ultimately propagates a series of action potentials across the retinal and brain neurons, and we perceive an image. The photoreceptors are dependent upon a well-functioning adjoining retinal pigment epithelium (RPE), which phagocytoses the spent photoreceptor outer segments that are shed daily to recycle photopigments (such as rhodopsin) and membrane lipids. The RPE also transports nutrients and breakdown products between the photoreceptors and the adjoining choroidal vascular plexus, separated from the RPE by Bruch’s membrane (Figures 1 and 2). Therefore the photoreceptors, choroidal vessels, and RPE have an interdependent relationship, and loss of any one component causes dysfunction of the others. This dysfunction is often seen first in the macula because the densely packed photoreceptors here have the highest oxygen consumption and metabolic rate of any tissue in the body (7).

In AMD the intimate relationship of RPE, photoreceptors, and choroid is interrupted
Clinically, AMD begins with the asymptomatic appearance of drusen, seen as white or yellow spots beneath the retina. What triggers the formation of drusen is not completely understood, and not all drusen formation leads to AMD. Drusen are deposits of lipid and cellular debris that are found beneath the RPE on Bruch’s membrane (Figures 1 and 2). These deposits increasingly separate the RPE from the underlying choroidal vascular bed, interrupting the function of the RPE and leading to photoreceptor degeneration, a hallmark of dry AMD (Figures 1 and 2). In wet AMD, its most devastating form, abnormal leaky choroidal vessels proliferate and penetrate the altered Bruch’s membrane protruding into the subretinal space, causing hemorrhage and rapid loss of vision.

Role of inflammatory cells in AMD
Many risk factors for AMD are related to inflammation. These include environmental factors, such as smoking and low omega-3 fatty acid intake (8, 9), and genetic factors, such as polymorphisms complement factor H (10) and CX3CR1 (2), the chemokine CX3CL1 receptor. Chemokines are small proteins that induce directed chemotaxis in nearby responsive immune cell populations, and CX3CL1 has been implicated in inflammation at many sites. The CX3CL1 receptor is expressed on a variety of immune cells, including T cells, macrophages, and microglia. CX3CR1 and CX3CL1 are both expressed on microglia, and CX3CL1 is upregulated in the retina of AMD patients (10-12). CX3CL1 is also upregulated in AMD patients with wet AMD, and CX3CL1 expression is higher in AMD patients than in normal controls (2).

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cells. Macrophage chemoattractants as well as elevated levels of inflammatory mediators and activated complement components are found in drusen samples from AMD patients (11, 12).

However, the mechanism of AMD with respect to inflammation and chemotactants is not well defined. In their current study, Combadière and coworkers (6) expand our understanding of the role of chemokines in AMD. They suggest that defective microglia with abnormal CX3CR1 function contribute to the formation of drusen in AMD and subsequently promote photoreceptor loss and choroidal neovascularization. The authors show that, compared with mice with normal microglia, mice deficient in CX3CR1 had subretinal accumulation of microglia and drusen-like deposits after insults to the retina including senescence, light-induced retinal degeneration, and laser damage to rupture Bruch’s membrane. The drusen-like material contained photoreceptor pigment (rhodopsin), indicating contribution from shed rod photoreceptor outer segments, as well as markers indicating contribution from accumulated defective microglia lacking CX3CR1 expression. Because these deposits precede photoreceptor degeneration and choroidal neovascularization (Figure 2), the authors suggest that these drusen could cause these AMD features. This study provides new insight into the putative role of microglia in drusen formation and into the role of inflammation in the pathogenesis of AMD, but raises many questions as well. How and why do these defective microglia accumulate — through decreased egress alone or increased attraction to the area? How do CX3CR1-defective microglia cause photoreceptor degeneration and choroidal neovascularization? How do these results reconcile with studies showing that decreased macrophage recruitment increases aspects of AMD?

Are microglia the cause or the cure for AMD?

In aged transgenic mice lacking CC chemokine ligand 2 (Ccl2) or its cognate receptor
CC chemokine receptor 2 (Ccr2), both molecules involved in chemotraction of macrophages and/or microglia, some features of AMD (drusen-like deposits and choroidal neovascularization) have been reported, suggesting that macrophage recruitment by Ccl2 or Ccr2 confers protection against the formation of AMD (13). Mice deficient in IL-10 — a potent inhibitor of cytokine and chemokine production — with inhibited recruitment of macrophages in retina have increased choroidal neovascularization (14). These results suggest that macrophage recruitment into the subretinal space is necessary to remove extracellular deposits and prevent the formation of drusen. Yet global depletion of macrophages and microglia in mice reduces laser-induced choroidal neovascularization (15). In contrast, the results reported here by Combadière and coworkers (6) suggest that accumulated CX3CR1-deficient microglia secondary to increased recruitment or impaired egress from the retina can form drusen and cause photoreceptor degeneration and choroidal neovascularization, all aspects of AMD.

CX3CR1 is involved in leukocyte recruitment, and previous studies have shown that its loss leads to inhibition of leukocyte migration (5). It is likely that an alternate chemoattractant molecule, like that of Ccr2, is involved in recruitment of these cells into areas of retinal damage. But how does accumulation of CX3CR1-deficient microglia cause AMD-like symptoms?

Perhaps it is because macrophages can have many different and even opposite functions depending on their activation state. Given this possibility, the results of the present study fit with the recent finding in muscle that decreased expression of CX3CR1 results in macrophages with a proinflammatory phenotype compared with macrophages with high levels of CX3CR1, which have an antiinflammatory phenotype (16). It is reasonable to speculate that the accumulation in the subretinal space of resident microglia with reduced CX3CR1 function might therefore contribute to low-grade inflammation, leading to the recruitment of other inflammatory cells including proangiogenic, bone marrow–derived macrophages, which in turn induce choroidal neovascularization and the pathology of AMD. Prolonged presence of these cells in the subretinal space may also spur phagocytosis of lipids normally processed by the RPE. These bloated microglia trapped subretinally might become dysfunctional and degenerate, contributing to the accumulation of extracellular cellular debris and to the early sign of drusen formation. Although the present study (6) raises interesting possibilities regarding the mechanism of drusen formation, the exact contribution of microglia and circulating macrophages to retinal degeneration and choroid neovascularization remains undefined.

Current treatment of AMD

The study by Combadière and coworkers (6) is an important contribution to the current inflammatory theory in AMD. A clear understanding of the cellular and molecular mechanisms of AMD could bring a major shift in our approach to disease treatment and prevention. Currently the only treatment to slow the progression of dry AMD to wet AMD is dietary supplementation with vitamins and antioxidants (17), which is consistent with the role of oxidation stress in this pathological process (18). Recently, higher intake of dietary lipids thought to be antiinflammatory (omega-3 long-chain polyunsaturated fatty acids and fish) (9) have been found to be associated with decreased likelihood of developing wet AMD (8). For wet AMD, the treatments focus on suppression of choroidal neovascularization, with laser ablation, photodynamic therapy, or, increasingly, anti-VEGF angiogenesis inhibitors. However, these treatments do not address the underlying cause of AMD, and preventive and nondestructive therapies are much more desirable.

A twin study by Seddon et al. found that genetic factors explain 46%–71% of the variation in AMD disease severity, while environment explains 19%–42% (19), so manipulation of some pathways involved in genetic risk could have significant benefit. But the possibility of manipulating the CX3CR1/CX3CL1 pathway specifically to prevent the development of AMD is uncertain at the moment. CX3CL1-deficient mice are protected against development of atherosclerosis (20), and CX3CL1 mediates antitumor responses (21), so manipulation of the CX3CR1/CX3CL1 pathway in AMD is complex and might disrupt the physiological process of microglia and/or macrophage recruitment and clearing of subretinal debris. Nevertheless, the study by Combadière and coworkers (6) suggests a new direction. Further understanding of the molecular basis of the pathogenesis of AMD might lead to new treatment based on inhibition of complement factors that promote choroidal neovascularization as well as pharmacological targeting of chemoattractant cytokines.

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Why targeted therapy hasn’t worked in advanced cancer

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In this issue of the JCI, Nissen et al. report that a reciprocal interaction exists between the growth factors FGF2 and PDGF-BB, causing tumors to exhibit increased angiogenesis and metastatic potential (see the related article beginning on page 2766). Both FGF2 and PDGF-BB signal through tyrosine kinase receptors, which have been the target of tyrosine kinase inhibitors for cancer therapies. These inhibitors are usually small molecules that inhibit the kinase activity of a receptor or nonreceptor tyrosine kinase, preventing downstream signaling. The results of this study shed light on why tyrosine kinase inhibitors have been useful for the treatment of only a small number of advanced cancers. Currently, a major focus of pharmaceutical companies is to develop ever more potent and specific tyrosine kinases. The results presented here suggest that this approach may not be successful.

Tyrosine kinases are a large family of enzymes that phosphorylate target proteins, resulting in either activation or inactivation of these proteins. This family includes many peptide receptors as well as nonreceptor proteins and is well represented in oncogenic fusion proteins, such as the BCR-ABL protein, which is generated from the Philadelphia chromosome. These proteins activate multiple signaling pathways, including those involving PI3K, phospholipase Cγ1, MAPK, and STAT activation and the generation of reactive oxygen species. The study by Nissen et al. in this issue of the JCI demonstrates that activation of a combination of several tyrosine kinase receptors results in a high-